

WE CLAIM:

1. A purified phenol oxidizing enzyme obtainable from *Stachybotrys*.
- 5 2. The phenol oxidizing enzyme of Claim 1 capable of modifying the color associated with a dye or colored compound.
3. The phenol oxidizing enzyme of Claim 1 wherein said enzyme exhibits an increase in apparent molecular weight after boiling, as determined by SDS-
10 polyacrylamide gel electrophoresis.
4. The phenol oxidizing enzyme of Claim 1 wherein the *Stachybotrys* includes *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisbyi*,
15 *S. cylindrospora*, *S. dichroa*, *S. oenanthae* and *S. nilagerica*.
5. The phenol oxidizing enzyme of Claim 1 wherein the *Stachybotrys* is *Stachybotrys chartarum* or *Stachybotrys parvispora*.
6. The phenol oxidizing enzyme of Claim 5 wherein the *Stachybotrys*
20 *parvispora* has MUCL accession number 38996.
7. The phenol oxidizing enzyme of Claim 5 wherein the *Stachybotrys* *chartarum* has MUCL accession number 38898.
- 25 8. The phenol oxidizing enzyme of Claim 1 having at least one antigenic determinant in common with phenol oxidizing enzyme obtainable from *Stachybotrys parvispora* MUCL accession number 38996 as measured by an immunoprecipitation line by Ouchterlony technique.
- 30 9. The phenol oxidizing enzyme of Claim 1 having at least one antigenic determinant in common with phenol oxidizing enzyme obtainable from *Stachybotrys*

chartarum MUCL accession number 38898 as measured by an immunoprecipitation line by Ouchterlony technique.

5 10. The phenol oxidizing enzyme of Claim 1 having an apparent non-denatured molecular weight of 38 kD as determined by SDS-PAGE.

11. The phenol oxidizing enzyme of Claim 1 having an apparent non-denatured molecular weight of 30.9 kD as determined by SDS-PAGE.

10 12. The phenol oxidizing enzyme of Claim 1, further characterized by having a pH optimum of from 5.0 to 7.0, inclusive, as determined by incubation for 2 minutes at 20 degrees C with ABTS as substrate.

15 13. The phenol oxidizing enzyme of Claim 1, further characterized by having a pH optimum of from 6.0 to 7.5, inclusive, as determined by incubation for 2 minutes at 20 degrees C with syringaldizin as substrate.

20 14. The phenol oxidizing enzyme of Claim 1, further characterized by having a pH optimum of from 7.0 to 9.0, inclusive, as determined by incubation for 2 minutes at 20 degrees C with 2,6-dimethoxyphenol as substrate.

25 15. A phenol oxidizing enzyme obtainable from *Stachybotrys* and having at least 65% identity to the phenol oxidizing enzyme having the amino acid sequence as disclosed in SEQ ID NO:2.

16. The phenol oxidizing enzyme of Claim 15 which has the amino acid sequence as disclosed in SEQ ID NO:2.

30 17. The phenol oxidizing enzyme of Claim 15 wherein said *Stachybotrys* includes *S.parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S.bisbyi*, *S.cylindrospora*, *S. dichroa*, *S. oenantes* and *S. nilagerica*.

18. An isolated polynucleotide encoding the phenol oxidizing enzyme of Claim 15.

19. An isolated polynucleotide encoding the amino acid having the sequence as shown in SEQ ID NO:2.

20. The isolated polynucleotide of Claim 18 having at least 65% identity to the nucleic acid having the sequence disclosed in SEQ ID NO: 1 or SEQ ID NO:3, or which is capable of hybridizing to the nucleic acid having the sequence disclosed in SEQ ID NO: 1 or SEQ ID NO:3 under conditions of intermediate to high stringency, or which is complementary to the nucleic acid having the sequence as shown in SEQ ID NO:1 or SEQ ID NO:3.

21. The isolated polynucleotide of Claim 20 having the nucleic acid sequence as disclosed in SEQ ID NO:1 or SEQ ID NO:3.

22. An expression vector comprising the polynucleotide of Claim 18, 19, 20 or 21.

23. A host cell comprising the expression vector of Claim 22.

24. The host cell of Claim 23 that is a filamentous fungus.

25. The host cell of Claim 24 wherein said filamentous fungus includes *Aspergillus* species, *Trichoderma* species and *Mucor* species.

26. The host cell of Claim 23 that is a yeast.

27. The host cell of Claim 26 wherein said yeast includes *Saccharomyces*, *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kluyveromyces*, and *Yarrowia* species.

28. The host cell of Claim 23 wherein said host is a bacterium.

29. The host cell of Claim 28 wherein said bacterium includes *Bacillus* and *Escherichia* species.

30. A method for producing a phenol oxidizing enzyme obtainable from *Stachybotrys* in a recombinant host cell comprising the steps of:

- 5 (a) obtaining a recombinant host cell comprising a polynucleotide encoding said phenol oxidizing enzyme obtainable from *Stachybotrys* wherein said enzyme has at least 65% identity to the amino acid sequence disclosed in SEQ ID NO:2;
- (b) culturing said host cell under conditions suitable for the production of said phenol oxidizing enzyme; and
- 10 (c) optionally recovering said phenol oxidizing enzyme produced.

31. A method for producing a phenol oxidizing enzyme, said method comprising the step of culturing a recombinant host cell, under suitable conditions, said host cell characterized by the expression of a polynucleotide encoding a phenol oxidizing enzyme obtainable from *Stachybotrys* wherein said enzyme has at least 65% identity to the amino acid having the sequence as shown in SEQ ID NO:2 and optionally recovering said phenol oxidizing enzyme.

15

32. The method of Claim 30 or Claim 31 wherein said phenol oxidizing enzyme is obtainable from a *Stachybotrys* including *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisayi*, *S. cylindrospora*, *S. dichroa*, *S. oenanthes* and *S. nilagerica*.

20

33. The method of Claim 30 or Claim 31 wherein said phenol oxidizing enzyme is obtainable from *S. chartarum* and has the amino acid sequence as disclosed in SEQ ID NO:2.

25

34. The method of Claim 30 or Claim 31 wherein said polynucleotide comprises the nucleic acid having the sequence as shown in SEQ ID NO:1 or SEQ ID NO:3 or is capable of hybridizing to the nucleic acid having the sequence as shown in SEQ ID NO: 1 or SEQ ID NO:2 under conditions of intermediate to high stringency, or is complementary to the nucleic acid having the sequence as shown in SEQ ID NO:1 or SEQ ID NO:3.

30

35. The method of Claim 30 or Claim 31 wherein said host cell includes filamentous fungus, yeast and bacteria.

36. The method of Claim 35 wherein said yeast includes *Saccharomyces*,
5 *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kluyveromyces*, and *Yarrowia* species.

37. The method of Claim 35 wherein said filamentous fungus includes *Aspergillus* species, *Trichoderma* species and *Mucor* species.

10 38. The method of Claim 36 wherein said *Saccharomyces* is *S. cerevisiae*.

39. The method of Claim 37 wherein the filamentous fungus is *Aspergillus niger* var. *awamori*.

15 40. The method of Claim 39 wherein said *Trichoderma* species is *Trichoderma reesei*.

6622E0" 256E2360
41. A method for producing a host cell comprising a polynucleotide encoding a phenol oxidizing enzyme obtainable from *Stachybotrys* said enzyme having at least
20 65% identity to the amino acid having the sequence disclosed in SEQ ID NO:2, said method comprising the steps of:

- (a) introducing a polynucleotide encoding said phenol oxidizing enzyme into a host cell; and
- (b) optionally culturing said host cell under conditions suitable for the
25 production of said phenol oxidizing enzyme.

42. The method of Claim 41 wherein said host cell includes filamentous fungus, yeast and bacteria.

30 43. The method of Claim 42 wherein said filamentous fungus includes *Aspergillus* species, *Trichoderma* species and *Mucor* species.

44. The method of Claim 43 wherein said *Aspergillus* species is *Aspergillus niger* var. *awamori*.

45. The method of Claim 43 wherein said *Trichoderma* species is
5 *Trichoderma reseei*.

46. The method of Claim 42 wherein said yeast is a *Saccharomyces* species.

47. The method of Claim 46 wherein said *Saccharomyces* species is
10 *Saccharomyces cerevisiae*.

48. The method of Claim 41 wherein said polynucleotide has at least 65% identity to the nucleic acid having the sequence as shown in SEQ ID NO:1 or SEQ ID NO:3, or is capable of hybridizing to the nucleic acid having the sequence as shown in SEQ ID NO:1 or SEQ ID NO:3 under conditions of intermediate to high stringency, or is complementary to nucleic acid having the sequence as shown in
15 SEQ ID NO:1 or SEQ ID NO:3.

49. The method of Claim 41 wherein said polynucleotide has the nucleic acid
20 sequence as shown in SEQ ID NO:1 or SEQ ID NO:3.

50. A recombinant host cell comprising a polynucleotide having at least 65% identity to the nucleic acid having the sequence as shown in SEQ ID NO:1 or SEQ ID NO:3, or which is capable of hybridizing to the nucleic acid having the sequence as shown in SEQ ID NO:1 or SEQ ID NO:3 under conditions of intermediate to high stringency, or which is complementary to nucleic acid having the sequence as shown
25 in SEQ ID NO:1 or SEQ ID NO:3.

51. The host cell of Claim 50 wherein said polynucleotide is present on a
30 replicating plasmid.

52. The host cell of Claim 50 wherein said polynucleotide is integrated in the host cell genome.

53. The recombinant host cell of Claim 50 which includes filamentous
5 fungus, yeast and bacteria.

54. A substantially pure culture of the strain *Stachybotrys parvispora* MUCL
38996.

10 55. A substantially pure culture of the strain *Stachybotrys chartarum* MUCL
38898.

15 56. An enzyme composition comprising the phenol oxidizing enzyme of
Claim 1.

20 57. The enzyme composition of Claim 56 wherein said phenol oxidizing
enzyme has at least 65% identity to the phenol oxidizing enzyme having the amino
acid sequence as disclosed in SEQ ID NO:2.

20 58. The enzyme composition of Claim 56 wherein said phenol oxidizing
enzyme has the amino acid sequence as disclosed in SEQ ID NO:2.